

CLAIMS

What is claimed is:

1. A DNA expression construct comprising, in 5' to 3' order: a promoter, the promoter operationally linked to a DNA sequence encoding streptokinase, wherein the expression construct drives formation of inclusion bodies comprising enzymatically-active streptokinase in a host cell transformed to contain the expression construct.
2. The DNA expression construct of Claim 1, wherein the promoter is a λ pR- λ pL promoter.
3. The DNA expression construct according to Claim 1, wherein the DNA sequence encoding streptokinase has a DNA sequence of SEQ. ID. NO. 3.
4. A method of producing streptokinase comprising transforming a host cell with an expression construct according to Claim 1, whereby the host cell expresses inclusion bodies comprising enzymatically-active streptokinase.
5. The method of claim 4, wherein the host cell is an *E. coli* cell.
6. The method of claim 4, further comprising transforming the host cell with another DNA expression construct having a BRP gene, wherein the BRP gene produces permeable zones in the host cell's cell envelope when activated, whereby enzymatically-active streptokinase expressed by the host cell is secreted through the permeable zones in the cell envelope.
7. The method of claim 4, further comprising heat-inducing the host cell, thereby increasing streptokinase production in the host cell as compared to streptokinase production in the host cell when the host cell is not heat induced.

8. The method according to Claim 4, further comprising:
inoculating culture media with the transformed host; and
fermenting the transformed host.

9. The method according to Claim 8, further comprising isolating the enzymatically-active streptokinase produced.

10. The method according to Claim 9, wherein the enzymatically-active streptokinase is isolated by steps comprising:
(a) pelleting the transformed host;
(b) disrupting the transformed host to release the inclusion bodies and partitioning the released inclusion bodies;
(c) isolating the partitioned inclusion bodies;
(d) solubilizing the isolated inclusion bodies;
(e) diafiltering the solubilized inclusion bodies;
(f) purifying the diafiltered inclusion bodies by ion exchange chromatography and then by gel permeation chromatography to separate fractions containing the streptokinase; and
(g) diafiltering the fractions containing the streptokinase.

11. A genetically-engineered host cell which expresses enzymatically-active streptokinase comprising: a host cell transformed to contain an expression construct comprising, in 5' to 3' order: a promoter, the promoter operationally linked to a DNA sequence encoding streptokinase, wherein the expression construct drives formation of inclusion bodies comprising enzymatically-active streptokinase in the host cell.

12. A DNA expression construct comprising, in 5' to 3' order: a promoter, the promoter operationally linked to a secretion signal sequence, the secretion signal sequence

operationally-linked to a DNA sequence encoding streptokinase, wherein the expression construct drives expression of enzymatically-active streptokinase in hosts transformed to contain the expression construct.

13. The DNA expression construct of Claim 12, wherein the promoter is a λ pR- λ pL promoter.
14. The DNA expression construct according to Claim 12, wherein the DNA sequence encoding streptokinase has a DNA sequence of SEQ. ID. NO. 3.
15. A method of producing streptokinase comprising transforming a host cell with an expression construct according to Claim 12, whereby the host cell expresses and secretes enzymatically-active streptokinase.
16. The method of claim 15, wherein the host cell is an *E. coli* cell.
17. The method of claim 16, further comprising heat-inducing the host cell, thereby increasing streptokinase production in the host cell as compared to streptokinase production in the host cell when the host cell is not heat induced.
18. The method according to Claim 17, further comprising:
inoculating culture media with the transformed host; and
fermenting the transformed host.
19. The method according to Claim 15, further comprising isolating the enzymatically-active streptokinase produced.
20. The method according to Claim 19, wherein the enzymatically-active streptokinase is isolated by steps comprising:

- (a) clarifying the supernatant containing the secreted streptokinase;
- (b) purifying the secreted streptokinase by ion exchange chromatography and then by gel permeation chromatography to separate fractions containing the streptokinase; and then
- (c) diafiltering the fractions containing the streptokinase.

21. A genetically-engineered host cell which expresses enzymatically-active streptokinase comprising a host cell transformed to contain and express an expression construct comprising, in 5' to 3' order: a promoter, the promoter operationally linked to a secretion signal sequence, the secretion signal sequence operationally-linked to a DNA sequence encoding streptokinase, wherein the expression construct drives expression of enzymatically-active streptokinase in hosts transformed to contain the expression construct.